AMENDMENTS TO THE SPECIFICATION:

Please amend the specification as shown below:

Please revise the paragraph beginning on page 33, line 5 as follows:

~~Figure 6~~ Figure 7. Distribution of saquinavir hyper-susceptibility by amino acid change at position 82.

Please revise the paragraph beginning on page 33, line 8 as follows:

~~Figure 7~~ Figure 8. Relative luciferase activity of integrase inhibitor-resistant site-directed mutants.

Please revise the paragraph beginning on page 33, line 11 as follows:

~~Fig. A~~ Figure 6A

Please revise the paragraph beginning on page 34, line 1 as follows:

~~Figure B.~~ Figure 6B

Please revise the paragraph beginning on page 34, line 14 as follows:

~~Figure C.~~ Figure 6C

Please revise the paragraph beginning on page 34, line 30 as follows:

~~Figure D.~~ Figure 6D

Please revise the paragraph beginning on page 35, line 8 as follows:

~~Figure E.~~ Figure 6E

Please revise the paragraph beginning on page 35, line 15 as follows:

~~Figure F.~~ Figure 6F

Please revise the paragraph beginning on page 35, line 22 as follows:

~~Figure G.~~ Figure 6G

Please revise the paragraph beginning on page 35, line 31 as follows:

~~Figure H.~~ Figure 6H

Please revise the paragraph beginning on page 36, line 8 as follows:

~~Figure I.~~ Figure 6I

Please revise the paragraph beginning on page 36, line 16 as follows:

~~Figure J.~~ Figure 6J

Please revise the paragraph beginning on page 36, line 24 as follows:

~~Figure K.~~ Figure 6K

Please revise the paragraph beginning on page 37, line 2 as follows:

~~Figure L.~~ Figure 6L

Please revise the paragraph beginning on page 37, line 8 as follows:

~~Figure M.~~ Figure 6M

Please revise the paragraph beginning on page 37, line 17 as follows:

~~Figure N.~~ Figure 6N

Please revise the paragraph beginning on page 37, line 29 as follows:

~~Figure O.~~ Figure 6O

Please revise the paragraph beginning on page 131, line 22 as follows:

Fitness tests were carried out with fitness test vectors using two host cell types. Fitness test vector viral particles were produced by a first host cell (the fitness test vector host cell) that was prepared by transfecting a packaging host cell with the fitness test vector and the packaging expression vector. The fitness test vector viral particles were then used to infect a second host cell (the target host cell) in which the expression of the indicator gene is measured ~~(see Fig. A)~~ (see Figure 6A).

Please revise the paragraph beginning on page 132, line 9 as follows:

The amount of luciferase activity detected in the infected cells is used as a direct measure of "infectivity", "replication capacity" or "fitness", i.e. the ability of the virus to complete a single round of replication. Relative fitness is assessed by comparing the amount of luciferase activity produced by patient derived viruses to the amount of luciferase activity

produced by a well-characterized reference virus (wildtype) derived, from a molecular clone of HIV-1, for example NL4-3 or HXB2. Fitness measurements are expressed as a percent of the reference, for example 25%, 50%, 75%, 100% or 125% of reference ~~(Figure B, C)~~ (Figure 6B, 6C).

Please revise the paragraph beginning on page 133, line 36 as follows:

Fitness test vectors were constructed as described in example 10. Fitness test vectors derived from patient samples or clones derived from the fitness test vector pools, or fitness test vectors were engineered by site directed mutagenesis to contain specific mutations, and were tested in a fitness assay to determine accurately and quantitatively the relative fitness compared to a well-characterized reference standard. A patient sample was examined for increased or decreased reverse transcriptase activity and correlated with the relative fitness observed ~~(Figure C)~~ (Figure 6C).

Please revise the paragraph beginning on page 131, line 2 as follows:

Reverse transcriptase activity can be measured .by any number of widely used assay procedures, including but not limited to homopolymeric extension using (e.g. oligo dT:poly rC) or real time PCR based on molecular beacons (reference Kramer) or 5'exonuclease activity (Lie and Petropoulos, 1996). In one embodiment, virion associated reverse transcriptase activity was measured using a quantitative PCR assay that detects the 5' exonuclease activity associated with thermo-stable DNA polymerases ~~(Figure C)~~ (Figure 6C). In one embodiment of the invention, the fitness of the patient virus was compared to a reference virus to determine the relative fitness compared to "wildtype" viruses that have not been exposed to reverse transcriptase inhibitor drugs. In another embodiment, the fitness of the patient virus was compared to viruses collected from the same patient at different timepoints, for example prior to initiating therapy, before or after changes in drug treatment, or before or after changes in virologic (RNA copy number), immunologic (CD4 T-cells), or clinical (opportunistic infection) markers of disease progression.

Please revise the paragraph beginning on page 135, line 21 as follows:

Genotypic changes that are observed to correlate with changes in fitness were evaluated by construction of fitness vectors containing the specific mutation on a defined, wild-type (drug susceptible) genetic background. Mutations may be incorporated alone and/or in combination with other mutations that are thought to modulate the fitness of a virus.

Mutations were introduced into the fitness test vector through any of the widely known methods for site-directed mutagenesis. In one embodiment of this invention the mega-primer PCR method for site-directed mutagenesis is used. A fitness test vector containing the specific mutation or group of mutations were then tested using the fitness assay described in Example 10 and the fitness was compared to that of a genetically defined wild-type (drug susceptible) fitness test vector which lacks the specific mutations. Observed changes in fitness are attributed to the specific mutations introduced into the resistance test vector. In several related embodiments of the invention, fitness test vectors containing site directed mutations in reverse transcriptase that result in amino acid substitutions at position 190 (G190A, G190S, G190C, G190E, G190V, G190T) and that display different amounts of reverse transcriptase activity were constructed and tested for fitness ~~(Figure D)~~ (Figure 6D). The fitness results were correlated with specific reverse transcriptase amino acid substituions and fitness.

Please revise the paragraph beginning on page 137, line 1 as follows:

Fitness test vectors were constructed as described in example 10. Fitness test vectors derived from patient samples or clones derived from the fitness test vector pools, or fitness test vectors engineered by site directed mutagenesis to contain specific mutations, were tested in a fitness assay to determine accurately and quantitatively the relative fitness compared to a well-characterized reference standard. A patient sample was examined further for increased or decreased protease activity correlated with the relative fitness observed ~~(Figure C)~~ (Figure 6C).

Please revise the paragraph beginning on page 137, line 18 as follows:

Protease activity of patient HIV samples Protease activity can be measured by any number of widely used assay procedures, including but not limited to in vitro reactions that measure protease cleavage activity (reference Erickson). In one embodiment, protease cleavage of the gag polyprotein (p55) was measured by Western blot analyis using an anti-capsid (p24) antibody ~~(Figure C)~~ (Figure 6C). In one embodiment of the invention, the fitness of the patient virus was compared to a reference virus to determine the relative fitness compared to "wildtype" viruses that have not been exposed to protease inhibitor drugs. In another embodiment, the fitness of the patient virus was compared to viruses collected from the same patient at different timepoints, for example prior to initiating therapy, before or after changes in drug treatment, or before or after changes in virologic (RNA copy number),

immunologic (CD4 T-cells), or clinical (opportunistic infection) markers of disease progression.

Please revise the paragraph beginning on page 138, line 19 as follows:

Genotypic changes that are observed to correlate with changes in fitness are evaluated by construction of fitness vectors containing the specific mutation on a defined, wild-type (drug susceptible) genetic background. Mutations may be incorporated alone and/or in combination with other mutations that are thought to modulate the fitness of a virus. Mutations are introduced into the fitness test vector through any of the widely known methods for site-directed mutagenesis. In one embodiment of this invention the mega-primer PCR method for site-directed mutagenesis is used. A fitness test vector containing the specific mutation or group of mutations are then tested using the fitness assay described in Example 10 and the fitness is compared to that of a genetically defined wild-type (drug susceptible) fitness test vector which lacks the specific mutations. Observed changes in fitness are attributed to the specific mutations introduced into the fitness test vector. In several related embodiments of the invention, fitness test vectors containing site directed mutations in reverse protease that result in amino acid substitutions at positions 30, 63, 77, 90 ~~(list from Figure E)~~ (list from Figure 6E) and that display different amounts of protease activity are constructed and tested for fitness ~~(Figure E)~~ (Figure 6E). The fitness results enable the correlation between specific protease amino acid substitutions and changes in viral fitness.

Please revise the paragraph beginning on page 140, line 32 as follows:

Reduced replication fitness was observed for a majority of the patient virus samples (Table A). Forty one percent of the viruses exhibited large reductions in replication fitness (<25% of the reference). Another 45% had moderate reductions (between 25-75% of the reference) in replication fitness. A minority of the patient samples (140) displayed replication fitness that approached or exceeded "wildtype" levels (>75% of the reference). Viruses with reduced drug susceptibility, were much more likely to display reduced replication fitness ~~(Figures F, G, H, and I)~~ (Figures 6F, 6G, 6H and 6I).

Please revise the paragraph beginning on page 141, line 13 as follows:

Greater than 10 mutations in protease were observed, in a majority of the patient virus samples (Table A) Viruses with reduced fitness were much more likely to contain 10 or more

protease mutations ~~(Figure I)~~ (Figure 6I). Sixty two percent of the viruses that exhibited large reductions in replication fitness (<25% of the reference) contained 10 or more protease mutations. Twenty two percent of the viruses with moderate reductions (between 25-75% of the reference) in fitness contained 10 or more protease mutations. Only 50 of the viruses that displayed replication fitness that approached or exceeded "wildtype" levels (>75% of the reference) contained 10 or more protease mutations (Table A). Certain protease mutations either alone (D30N) or in combination (L90M plus K20T, or M46I, or 73, or N88D) were observed at high incidences in viruses with reduced fitness ~~(Figures I and J)~~ (Figures 6I and 6J).

Please revise the paragraph beginning on page 141, line 31 as follows:

Reduced protease processing of the p55 gag polyprotein was observed in a majority of the patient virus samples (Table A). Viruses with reduced fitness were much more likely to display reduced protease processing; defined as having detectable amounts of the p41 intermediate cleavage product ~~(Figures F, I and K)~~ (Figures 6F, 6I and 6K). Seventy one percent of the viruses that exhibited large reductions in replication fitness (<25% of the reference) displayed reduced protease processing. Eighteen percent of the viruses with moderate fitness reductions (between 25-75% of the reference) displayed reduced protease processing. Only 10% of the viruses that displayed replication fitness that approached or exceeded "wildtype" levels (>75% of the reference) exhibited reduced protease processing (Table A). Certain protease mutations (D30N, M46I/L, G48V, I54L/A/S/T/V, and I84V) were observed at high incidences in viruses with reduced protease processing of the p55 gag polyprotein ~~(Figure L)~~ (Figure 6L).

Please revise the paragraph beginning on page 144, line 8 as follows:

Of the 18 patients that interrupted therapy, 16 patients had resistant viruses that regained susceptibility to antiretroviral drugs during the period of treatment interruption. The phenotypic test results of a representative patient are shown in ~~Figure M~~ Figure 6M. Typically, susceptibility returned to all drug classes simultaneously, consistent with the re-emergence of a minor population of drug sensitive virus. In the representative example shown in Figure M, drug sensitivity was abruptly restored between weeks 9 and 10. Genotypic analysis (DNA sequence of protease and reverse transcriptase) are also consistent with the re-emergence of a drug sensitive virus. These data show the loss of most or all drug resistance mutation simultaneously (data not shown). The data are not consistent with random

back mutations. Back mutations would predict that restored susceptibility to drugs would occur unevenly for different drug classes and/or within a drugs within the same class.

Please revise the paragraph beginning on page 144, line 27 as follows:

Generally, the re-emergence of the drug susceptible virus was also accompanied by a simultaneous increase in replication fitness. This relationship is clearly evident for the representative virus ~~(Figure N)~~ (Figure 6N). Several other examples with less frequent timepoints are shown in Figure O. Virus from patients that did not revert to drug susceptibility after interruption generally did not exhibit an increase in replication fitness, nor did viruses from patients that did not interrupt treatment ~~(Figures O)~~ (Figure 6O). The data indicate that the drug sensitive virus that re-emerged after treatment interruption is able to replicate better than the drug resistant virus that was present before treatment was interrupted. The re-emergence of drug susceptible virus in this group of patients was also accompanied by an increase in viral load and a decrease in DC4 T-cells, indicators of disease progression. Thus, fitness information can be used to guide treatment of patients that harbor multi-drug resistant virus and are considering treatment interruption. If the patient virus is drug resistant but has low replication capacity, the patient and the physician should consider continuing drug treatment to prevent the re-emergence of a drug sensitive virus with higher replication capacity and greater pathogenecity. Alternatively, if the patient virus is drug resistant and has high replication capacity, the patient and the physician may consider interrupting treatment to spare the patient from the harmful and unpleasant side effects of antiretroviral drugs that are not providing clinical benefit.

Please revise the paragraph beginning on page 148, line 29 as follows:

In a preferred embodiment of this invention, evaluation of the effects of mutations at amino acid position 82 of HIV-1 protease on protease inhibitor susceptibility, was carried out using a phenotypic susceptibility assay using resistance test vector DNA prepared from the biological sample. In one embodiment, plasma samples were collected, viral RNA was purified and an RT-PCR methodology was used to amplify a patient derived segment encoding the HIV-1 protease and reverse transcriptase regions. The amplified patient derived segments were then incorporated, via DNA ligation and bacterial transformation, into an indicator gene viral vector thereby generating a resistance test vector. Resistance test vector DNA was isolated from the bacterial culture and the phenotypic susceptibility assay was carried out as described in Example 1. The genotype of the protease region was determined

by dideoxy chain- termination sequencing of the resistance test vector DNA. The results are summarized for saquinavir (SQV) in ~~Figure 6~~ Figure 7. Samples were categorized as having mutations in protease encoding alanine (A), phenylalanine (F), or threonine (T) at position 82, instead of the wild-type valine (V), and the percentage of samples in each category displaying hyper-sensitivity to saquinavir (i.e., fold-change vs. reference of 0.4 or less) was determined. Surprisingly, the percentage of saquinavir hyper-susceptible viruses was much higher amongst viruses containing V82F than those containing V82A or V82T. This observation implies that the detection of V82F in protease predicts a positive virological response to saquinavir treatment.

Please revise the paragraph beginning on page 150, line 33 as follows:

Genotypic changes that are observed to correlate with resistance to integrase inhibitors are evaluated by construction of fitness vectors containing the specific mutation on a defined, wild-type (drug susceptible) genetic background. Mutations may be incorporated alone and/or in combination with other mutations that are thought to modulate the fitness of a virus. Mutations are introduced into the fitness test vector through any of the widely known methods for site-directed mutagenesis. In one embodiment of this invention the mega-primer PCR method for site-directed mutagenesis is used (Sarkar, G. and Sommar, S.S., 1994, Biotechniques 8, 404-407). A fitness test vector containing the specific mutation or group of mutations are then tested using the fitness assay described in Example 10 and the fitness is compared to that of a genetically defined wild-type (drug susceptible) fitness test vector which lacks the specific mutations. Observed changes in fitness are attributed to the specific mutations introduced into the fitness test vector. In several related embodiments of the invention, fitness test vectors containing site directed mutations in integrase that result in amino acid substitutions at positions 66, 154, 66 and 153, and 66 and 154 are constructed and tested for fitness ~~(Figure 7)~~ (Figure 8). As controls, mutants with multiple changes conferring resistance to reverse transcriptase and protease inhibitors (MDRC4) and with a mutation in the integrase active site (D64V) were also tested. The fitness results enable the correlation between specific integrase amino acid substitutions and changes in viral fitness.